Molecular characterization and sex-specific tissue expression of prolactin, somatolactin and insulin-like growth factor-I in yellow perch (*Perca flavescens*)

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Abstract

The cDNA sequences encoding prolactin (PRL), somatolactin (SL) and insulin-like growth factor-I (IGF-I) genes of the yellow perch were obtained. Brain, pituitary, gill, heart, liver, stomach, kidney, spleen, muscle and gonad tissues were analyzed from both male and female adult yellow perch for sex-specific tissue expression. The full length cDNA of yellow perch PRL consists of 2306 bp and PRL expression was highest in the yellow perch pituitary with low to moderate expression in other tissues including brain, gill and post-vitellogenic oocytes. The full length cDNA of yellow perch SL consists of 1589 bp and SL expression was highest in the yellow perch pituitary with low to moderate expression in other tissues including brain, gill, liver, stomach, spleen and kidney. The full length cDNA of yellow perch IGF-Ib consists of 814 bp and tissue expression analysis of yellow perch IGF-I revealed a second yellow perch transcript (IGF-Ia) that is 81 nucleotides smaller. Both IGF-Ib and IGF-Ia had the greatest expression in liver tissue with moderate expression in brain, spleen and kidney tissues of both sexes. These sequences are valuable molecular tools which can be used in future studies investigating the basis for sexually dimorphic growth in yellow perch.
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1. Introduction

Prolactin (PRL) is a protein hormone in the growth hormone (GH), somatolactin (SL) and placental lactogen (PL) family which is thought to have resulted from gene duplication some 400 million years ago (Miller and Eberhardt, 1983). The primary site of PRL production in all known vertebrates is the anterior pituitary gland but recent work has highlighted extrapituitary PRL production (Ben-Jonathan et al., 1996). In teleosts, PRL expression has also been demonstrated in numerous peripheral tissues (e.g., brain, gill, intestine, spleen, liver, ovary and testes) (Zhang et al., 2004; Imaoka et al., 2000; Santos et al., 1999). Tissue distribution of the PRL receptor in teleosts (e.g. Lee et al.,

2006) is consistent with the primary function of PRL as a freshwater adapting hormone, however the full spectrum of PRL functions, in teleosts, humans or otherwise, is not completely understood (Bole-Feysot et al., 1998). Shepherd et al. (1997) found PRL$_{177}$ elevated IGF I mRNA in tilapia (*Oreochromis mossambicus*), presumably by acting as a competitive ligand for GH receptors and Riley et al. (2002) showed that ghrelin, a peptide which specifically stimulates GH release from the pituitary, significantly stimulated PRL release in tilapia. Leena et al. (2001) showed that PRL inhibited several enzymes involved in biosynthesis of fatty acids in climbing perch (*Anabas testudineus*) liver and PRL treatment in juvenile coho salmon (*Onchorhynchus kisutch*) resulted in pronounced lipid depletion (Sheridan, 1986). These findings indicate at least a peripheral somatotrophic action of PRL in teleosts.

Somatolactin (SL) is the newest member of the GH/PRL/SL/PL family of hormones (Ono et al., 1990) but has only been found in fish. SL is primarily produced in the pars intermedia of

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Table 1

Forward (F) and reverse (R) primer sequences used to generate initial PCR products for PRL, SL, and IGF-I

Gene		Start	Primer sequence
	F	–	GeneRacer™ 5′ primer
PRL	R	543	AGG GCA GTG AGG AGA TGG CCT GAG C
	F	–	GeneRacer™ 5′ primer
SL	R	658	GGG CGT CTT TCT TGA AGC AGC TGA G
	F	–	GeneRacer™ 5′ primer
IGF-I	R	592	TCT GGC TGC TGT GCT GTC CTA CG

the pituitary gland but at least one study has found extrapituitary expression in brain, gill, heart, kidney, liver, skeletal muscle, spleen, ovary and testis (Yang et al., 1997). SL has been tied to a number of physiological processes which include, but are not limited to, smoltification (Rand-Weaver and Swanson, 1993), stress response (Rand-Weaver et al., 1993), gonadal steroid biosynthesis (Planas et al., 1992) and gonadal maturation (Rand-Weaver et al., 1992). Fukada et al. (2005) found the highest levels of SL receptor expression in liver, visceral fat, muscle and gonad in both sexes of chum salmon. SL deficient mutant (*ci*) medaka had significantly higher hepato-somatic index (HSI), muscle triglycerides, liver triglycerides and lower plasma cortisol levels (Fukamachi et al., 2005) than the wildtype medaka. And a "cobalt" variant of rainbow trout which lacks most of the pars intermedia and is essentially SL deficient, showed similar patterns in triglycerides and cortisol levels (Yada et al., 2002). Moreover, SL was able to stimulate hepatic insulin-like growth factor I (IGF-I) expression *in vivo* in coho salmon (Duan et al., 1993). These studies and others (Vega-Rubín de Celis et al., 2004; Vega-Rubín de Celis et al., 2003) give clear evidence that SL is involved in energy homeostasis, lipid metabolism and possibly growth.

Insulin-like growth factors (IGFs) are mitogenic peptide hormones produced by all known vertebrates, mainly in the liver. Two separate IGFs (IGF-I and IGF-II) have been identified in bony fish and have been shown to mediate the growth-promoting actions of pituitary growth hormone (GH) (Reinecke et al., 2005; Duan, 1997). While the liver is the primary site of IGF-I expression in teleosts, numerous studies have found substantial non-hepatic expression in other tissues (e.g., brain, pituitary, gill, heart, stomach, spleen, kidney, muscle and gonad (some recent examples include (Caelers et al., 2004; Jentoft et al., 2004)). There is abundant evidence supporting the role of IGF-I in teleost growth and indicating that GH is the primary regulator of IGF-I synthesis and secretion (Biga et al., 2005; Carnevali et al., 2005). The E domain of IGF-I is highly variable and four different forms have been found in several salmonids which are designated IGF-I Ea-1, Ea-2, Ea-3, and Ea-4 (Duguay et al., 1994). The four transcripts are derived from two separate duplicated IGF-I genes (Kavsan et al., 1994) with two alternatively spliced transcripts from each duplicated gene (Wallis and Devlin, 1993). Eurasian perch, a close relative of yellow perch, has two IGF-I transcripts designated IGF-Ib (the larger one) and IGF-Ia (the smaller one) (Jentoft et al., 2004). Eurasian perch IGF-Ib shows high homology to coho salmon Ea-4, whereas Eurasian perch IGF-Ia corresponds with salmon Ea-2 (Duguay et al., 1994).

The yellow perch is one of a group of important fishes which exhibit a sexual size dimorphism (SSD) in which females grow faster than males. Studies have identified 17β-estradiol (E_2) as a growth stimulator in yellow perch SSD (Malison et al., 1988; Malison et al., 1986; Malison et al., 1985) and Roberts et al. (2004) found that E_2 increased pituitary GH content. In a later experiment, Jentoft et al. (2005) showed that administration of E_2 in juvenile yellow perch stimulated growth but neither bovine GH (bGH) nor recombinant yellow perch GH (rypGH) stimulated growth at a range of doses. Further, serum IGF-I levels in all experimental animals (E_2, bGH and rypGH treated) were not significantly different from IGF-I levels in control animals. Malison et al. (1988) reported that in addition to a growth response, E_2 treatment stimulated food consumption, and in another study (Hayward and Wang, 2001) growth rate and growth efficiency of female yellow perch exceeded those of males two-fold in animals fed without restriction. These observations suggest that the growth-promoting effects of estrogen may work, in part, through appetite centers of the central nervous system and could involve pituitary hormones, which alter appetite in teleosts (Johansson et al., 2005; Johnsson and Björnsson, 1994).

Pituitary function is controlled via a number of neuroendocrine factors, steroids (estrogen and testosterone) and the insulin family of polypeptides (e.g., IGF-I), suggesting the presence of an intricate, sex-specific (steroid), regulatory relationship between the pituitary, central nervous system and peripheral tissues (e.g., liver and gonads) (Melamed et al., 1996, 1995). As a first step in investigating the roles of PRL, SL and IGF-I in the estrogen stimulated SSD of yellow perch, the full length cDNAs for these genes were cloned and sequenced and their sex specific tissue expression was examined. Additionally, the deduced amino acid sequences translated from the yellow perch cDNA sequences were used to generate alignments and phylogenetic trees for each individual gene with all known teleost sequences providing an analysis of phylogenetic relationships.

2. Materials and methods

2.1. Cloning and sequence analyses

Pituitary and liver tissues were collected from adult yellow perch (*Perca flavescens*) brought into the Pannuzzo Fish Co.

Table 2

Forward (F) and reverse (R) primer sequences and size (bp) of the resulting full length cDNA coding region PCR products for GH, PRL, SL and IGF-I

Gene		Start	Primer sequence	Size (bp)
	F	36	CTC AAC CAG AAC TCA ACC AGA ACC AG	
GH	R	714	GAT GGA GGG GCA GGG CTA CAT GAT	678
	F	32	AGG CCA ACA AAC AGG AGG CAG AGA A	
PRL	R	726	AAG AAA ATC ATG CCG GCC GCC TCA C	694
	F	22	GAC AGA ATG CAC ATG GTG ACA G	
SL	R	789	AGT GCA CGG CTA CCA ACC TTA GGA A	767
	F	118	CGG GCT TTG TCT TGC GGA GAC C	
IGF-I	R	738	GTC GCT GGG CAT TTG TCC ATT CCT T	620

(Lorain, OH, USA) by recreational fishermen for cleaning. Tissues were collected from live fish, immediately frozen on dry ice, transported back to the University of Kentucky and stored at −80 °C until total RNA was extracted with the GenElute™ Mammalian Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA). RNA samples were treated with amplification grade DNase I (Sigma) and quantified on a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). First-strand cDNA with ligated 5′ and 3′ anchor primers was generated from 5 μg total RNA using the GeneRacer™ Kit (Invitrogen, Carlsbad, CA, USA).

GenBank was searched for neoteleost PRL, SL, and IGF-I cDNAs and several sequences were aligned using Vector NTI Suite 7.0 (Informax, Inc., Frederick, MD, USA) and GeneDoc (Nicholas et al., 1997). Consensus sequences, or fragments thereof, were entered into Primer3, a web-based primer design program (Rozen and Skaletsky, 2000) and general neoteleost primers for genes of interest were generated. Generated primers were used in combination with the GeneRacer™ 5′ primers (Table 1) provided in the GeneRacer™ Kit (Invitrogen) to obtain initial PCR products. A 50 μL total volume PCR mixture using a MasterTaq Kit (Eppendorf Scientific Inc., Westbury, NY, USA) was subjected to touch down PCR amplification with the following conditions: an initial denaturing step of 94 °C for 2 min; 5 cycles consisting of denaturing at 94 °C for 30 s, annealing at 72 °C for 30 s and extension at 70 °C for 60 s; 5 cycles consisting of denaturing at 94 °C, annealing at 70 °C and extension at 70 °C; 30 cycles consisting of denaturing at 94 °C, annealing at 68 °C, and extension at 70 °C; and a final extension step at 70 °C for 10 min. PCR products were electrophoresed in 1% agarose gels with a 1 kb DNA ladder (Gibco/BRL, Gaithersburg, MD, USA) and visualized by ethidium bromide staining. PCR products of expected size were electrophoresed in 1% low melt agarose gels, excised and purified using GenElute™ Minus EtBr Spin Columns (Sigma). Purified PCR products were ligated into a pCR®4-TOPO® vector and transformed into TOP10 chemically competent cells using the TOPO TA Cloning® Kit for Sequencing (Invitrogen). Plasmid DNA was extracted from the bacterial cells using the GenElute™ Plasmid Miniprep Kit (Sigma). Plasmid samples were quantified and up to 600 ng of plasmid DNA was used for PCR sequencing using BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Products were sequenced at the University of Kentucky Advanced Genetic Technologies Center.

Species-specific PRL, SL and IGF-I primers were developed based on the sequences generated and the PCR, cloning, and sequencing procedure was repeated as necessary (with 5′ and/or 3′ GeneRacer™ primers) to achieve full length (5′UTR+CDS+ 3′UTR) sequences. BLASTN v. 2.2.14 (Altschul et al., 1997)

Fig. 1. Nucleotide and deduced amino acid sequences of yellow perch PRL. The nucleotides (lower row) are numbered on the left and the amino acids (upper row) are numbered on the right. The signal peptide is underlined and the four cysteine residues in the mature protein are shaded while the putative polyadenylation signal is in bold and double underlined. Yellow perch PRL cDNA sequence is available from the EMBL/GenBank database with accession AY332491.

searches were used to determine similarities with other teleosts and to verify gene identity. To amplify the full coding region of yellow perch GH (Accession AY007303) (Roberts et al., 2004), PRL, SL and IGF-I cDNAs new primers were designed (Table 2). Total RNA (750 ng) extracted from pituitary (PRL and SL) or liver (IGF-I) tissues was reverse transcribed using the iScript™ cDNA Synthesis Kit (BioRad, Hercules, CA, USA) to generate cDNA templates. A 50 μL total volume PCR mixture using 1 μL of cDNA template was subjected to touch down PCR amplification under the following conditions: an initial denaturing step of 94 °C for 2 min 5 cycles consisting of denaturing at 94 °C for 30 s, annealing at 72 °C for 30 s and extension at 72 °C for 60 s; 5 cycles consisting of denaturing at 94 °C, annealing at 68 °C and extension at 72 °C; 30 cycles consisting of denaturing at 94 °C, annealing at 64 °C, and extension at 72 °C; and a final extension step at 72 °C for 10 min. PCR products were electrophoresed in 1% low-melt agarose gels and resulting bands were excised, purified, cloned and sequenced, as described above, to verify sequence data and primer-gene specificity. GeneDoc (Nicholas et al., 1997) was used to generate amino acid sequence alignments of PRL, SL and IGF-I from teleost sequences given by a BLASTP v. 2.2.14 search (Altschul et al., 1997). Alignments were produced in Clustal X1.81 (Thompson et al., 1994) and used to generate rooted phylogenetic tree data for the mature proteins using the Neighbor Joining tree method (Saitou and Nei, 1987), with 1000 bootstrap trials, and TreeView v. 1.61 was used to create a visual phylogenetic tree.

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                                     *        20        *        40        *        60        *        80        *       100        *
yellow perch               : MAHR-KTNGSKIFMTVL-YMVAACRAVAINDLLDRASERSDIMHSLSTTLSHDLDSQFPPIGRVM-PRPSMCHTASLQTPIDKYQALQVSESDLLSLARSLLQAWEDPLA : 107
orange-spotted grouper (85%) : ..Q.-HSD.N.LL....-.....S..P........Q...RL.........FN.....MAM....S....S..E..............A...V : 108
silver seabream        (81%) : ...-E.....L.II..-C.....G..P........Q...TL.........TK..SNHV..V.WTVM....L...S....N..A.....M.......R.... : 108
four-spine sculpin     (81%) : ...CGG.....F.IA.M-....GGIP.S.......Q...KL.........TQ...H.....IM..T...S....N..E...........V.........M : 109
bluefin tuna           (80%) : ...-G.....L....-.T.T..S.IP.S......QH..KL....I.TQE..H.....IT..A..S..E..N..E.......N..........A...V : 108
European sea bass      (80%) : ..Q.-.....L..M..-.....S.IP.S.......Q...TL.........TQ.....H...M..IT.....S.H.....E........A.....V....R...V : 108
red seabream           (80%) : ...-G.....L....I..-CI....S..P.......Q...TL.........TQ..NNHV..WM.M...L..S....N..E.T..L............Q...V : 108
gilthead seabream      (79%) : ....-E.....L.I...-C.....S..P.......Q...ML.........TK..SNHV..V.WT.M..PL..S....N..E......L........Q...V : 108
black seabream         (79%) : ..R.-E.....L.I..-C.....G..P.S......Q...TL.........TQ..NNHI..V.WM.M....T..S....N..E......L...........Q...V : 108
three spot gourami     (78%) : ..Q.-G....V.VA..-..A.V.G..HVS......Q...KL.........TQEM..H...LS.AFM.......S..M..N..E...............S.V..L : 108
Mozambique tilapia     (75%) : ..Q.-R.S.TNL.....-CV..M....P..E.FE...QH..KL.........TQE...H.....IM..A.....S.........D..........M.........V : 108
Nile tilapia           (75%) : .RQ.-R.S.TNL.....-CV..M....P..E.FE...Q..KL...S..TQE...H.....IM..A.....S.........D..........M.........V : 108
bastard halibut        (75%) : .T...-R.KLFMMAAV.S-.VMTS.G..P........Q...QL.........QE...H.....IM.........SA...N..T........E..........A...S : 108
pufferfish             (69%) : ..D.-RPSSIVL....WGVALTV.GG.STG..........LI....V..Q....HL.SL...HV........S....M..E........K............A...L : 109
Arctic cisco           (65%) : ..R.SQGTK---LHLAVLCL.VS.H.IGLS..ME...Q...KL.....S.TK...H...M.....M..S....K..E..R...NE.I....L..N..L : 107
rainbow trout          (65%) : ..R.SQGTK---LHLAVLCL.VS.H.IGLS..ME...Q...KL.....S.TK...H...M.....M..S....K..E..K...NE.I....L..N..L : 107
Atlantic salmon        (65%) : ..R.SQGTK---LHLAVLCL.VS.H.IGLS..ME...Q...KL.....S.TK...H...M.....M..S....K..E..R...NE.I....L..N..L : 107
Chinook salmon         (64%) : ..R.SQGTK---LHLAVLCL.VS.H.IGLS..ME...Q...KL.....S.NK...H...M.....M..S....I.K..E..R...NE.I....L..N..L : 107
chum salmon            (64%) : ..R.SQGTK---LHLAVLCL.VS.H.IGLS..ME...Q...KL.....S.TK...H...M.....M..S....K..E..K...NE.I....L..N..L : 107
stinging catfish       (63%) : ..RCC.CPRLHLAV...ACVLVFTEG.NL......QL..K.....S.TN...H.SSV.GKLM-........S...I.N..D...S.P.GE....V...M..S.... : 109
channel catfish        (61%) : ..RCC.CPRLHLAV...ACVLVFTEG.NL......QL..K.....S.TN...H.SSV.GKLM-........S...I.N..D...S.P.GE....V...M..S.... : 109
noble carp             (58%) : ..---EGSRLYFAV...MCAFVSING.GL....E...QL..KL....S.TN...H...V.....M..S...I.N..D...K.P.DE........L..S.... : 107
common carp            (57%) : .T---QGSRLYFAV...MCAFVSING.GL....E...QL..KL....S.TN...H...V.....M..S...I.N..D...KIP.DE.........L..S.... : 107
goldfish               (57%) : .T---QGSRLYFAVA..MCGFVSING.GL....E...QL..KL....S.TN...H...V.....M..S...I.N..D...K.P.DE.........L..S.... : 107
zebrafish              (57%) : .---QGSRQYFAVAI.MCAFVSING.GL....QL..KL.Y...S.TN...H...G.M...L..S...I.N..D...MK.P.DE........L..S.... : 107
Japanese eel           (57%) : ..Q.F.GSN--L.L.A.LCLASQGH..GLG.M.E...QL..KL....S.TN...TH...M.KILM........H..D...R.P..E.....A..LS.N...L : 108
European eel           (56%) : ..Q.F.GRS--L.L.A.LCLASQGY..GLG.M.E...QL..KL....S.TN...TH...M.KILM........H..D...R.P..E..I..A..LS.N...L : 108


                              120        *       140        *       160        *       180        *       200        *          .
yellow perch               : VLSTSANTLPHPAQSSISNKIQELQEHSKNLGDGLDILSGKMGPAAQTIASLPYRGGNDIGEDRISKLINFNLLSCFRRDSHKIDSFLKILRCRAAKMQPEMC : 211
orange-spotted grouper     : ...............T..........T.S..........L.S........Q.....V..........V......LR.... : 212
silver seabream            : D..N....S.L..S.........R.......S............S....AS.....N....T..H.......V.......V... : 212
four-spine sculpin         : .............A..........Y.S....VV..D..A.....SL..S.....Q..T.Q...........V........Q.L.. : 213
bluefin tuna               : ...A...S.....N.....V..........N....E.M.....S....-.N..Q.E.........V......QL... : 211
European sea bass          : I..........N...T.V...L..T.S.........F.....S.S..........SQ....R..T..H..M..........V......L..... : 212
red seabream               : D.......S.L..S.T.......R.............R.....A.S.........G....Q.T..H.......V........V... : 212
gilthead seabream          : D..N....S.L..S.........R.......S............V....A.S.........S......N....T..H.......V.......V... : 212
black seabream             : N..S.V..S.........R.......S............E..A.S.........S.....T..H........NV.......L.... : 212
three spot gourami         : ...V..S.................T.D..........E..A.SM.......Q....T..A.H..........V........I..L.. : 212
Mozambique tilapia         : ...S..S.........T.F....M.QY..S.K...V..S...SP..A.T........TNL.H.K.T...........L.......V.......... : 212
Nile tilapia               : ...S..S.........T.F....M.QY..S.K...V..S...SP..A.T........TNL.H.K.T...........L.......V.......... : 212
bastard halibut            : A..S..FS.........F..VR.M..........E...ALS....F..-..V.Q.......H.......V.......NT.... : 211
pufferfish                 : F..N.VS...QT...NA........KD...I......V..AR..Q...A.S....T...L.Q.KL...V.H...............M.N.L.... : 213
Arctic cisco               : L..SE.P....SNGD...S..R...DY..S.........VN.....SS.Y.S.I.FK.-G.L.N.KT.R...H..M..........V.......T..R..T. : 210
rainbow trout              : L..SE.P....SNGD...S..R...DY..S.........MVN.....SS.Y.S.I.FK.-G.L.N.KT.R...H..M..........V.......T..R..A. : 210
Atlantic salmon            : L..SE.P....SNGD...S..R...DY..S.........MVN.....SS.Y.S.I.FK.-G.L.N.KT.R...H..M..........V.......T..R..T. : 210
Chinook salmon             : L..SE.P....SNGD...S..R...DY..S.........VN.....SS.Y.SLI.FK.-G.L.N.KT.R...H..M..........V.......T..R..T. : 210
chum salmon                : L..SE.P....SNGD...S..R...DY..S.........MVN.....SS.Y.S.I.FK.-G.L.N.KT.R...H..M..........V.......T.IR..T. : 210
stinging catfish           : L..SE.TS....ERN..NT.TR...D.TST...A...R.VR...SSSESLS...FN.-..L.Q.NV.R.V..H..............V........L.D.. : 212
channel catfish            : L..SE.TS....ERN..NT.TR...D.TNS...A...ER.GR...SSPESLS...FNS-..L.Q.N..R.V..H..............V...D...L... : 212
noble carp                 : L..SE.SS.A..ERNT.NS.TK...DNINS...A..ERVVH...SSSDNLS...FYS-..SL.Q.KT.R.V..H..............V........KR.... : 210
common carp                : L..SE.SS.A..ERNT.DS.TK...DNINS...A..EHVFQ...SSSDNLS...FYT-SSL.Q.KT.R.V..H..............V........KR.... : 210
goldfish                   : L..SE.SS.A..ERNT.DS.TK...DNINS...A..EHVFN..DSTSDNLS...FDI-.SL.Q.KT.R.V..H..............V........KR.... : 210
zebrafish                  : L..SE.SS.A..ERNT.NS.TK...DNINS...A..EHVVH...SSSDNLST..FN.-.NL.Q.KT.R.V..H..............V......KR.D.. : 210
Japanese eel               : L.TSE.P..S..QNGV.YS.TR...DQ.NS.SS...R.IH.I.SSSKSLSP...FQ.-G.L.S.KN.R.....Y............N...L....Q--DR.. : 209
European eel               : L.ASE.P..S..QNGA.YS.TR...DQ.NS.SS...R.IH.I.SSSKALSP...LQ.-G.L.S.KN.R.....Y............N...L....Q--DR.. : 209
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Fig. 2. Alignment of the deduced yellow perch pre-pro-PRL amino acid sequence with other teleost pre-pro-PRLs. Conserved amino acid residues are indicated with a (.), inserted gaps are indicated with a (–), and conserved cysteine residues in the mature peptide are shaded. The percent listed (%) after the name for each sequence represents the homology with the yellow perch PRL sequence as determined by a BLASTP v. 2.2.14 search (Altschul et al., 1997). Sequences were downloaded from the EMBL/GenBank database with the following accession numbers: orange spotted grouper (*Epinephelus coioides*) AAO11695; silver seabream (*Rhabdosargus sarba*) ABB17072; gilthead seabream (*Sparus aurata*) CAD52820; black seabream (*Acanthopagrus schlegelii*) AAX21764; red seabream (*Pagrus major*) BAE43854; European sea bass (*Dicentrarchus labrax*) CAA55369; four-spine sculpin (*Cottus kazika*) BAE19673; three spot gourami (*Trichogaster trichopterus*) AAX09323; pufferfish (*Tetraodon nigroviridis*) AAR25696; Mozambique tilapia (*Oreochromis mossambicus*) CAA63124; Nile tilapia (*Oreochromis niloticus*) AAA53281; bastard halibut (*Paralichthys olivaceus*) AAD15746; bluefin tuna (*Thunnus thynnus*) BAE45636; goldfish (*Carassius auratus*) AAT74865; common carp (*Cyprinus carpio*) CAA37063; noble carp (*Hypophthalmichthys nobilis*) CAA43383; zebrafish (*Danio rerio*) AAH92358; Arctic cisco (*Coregonus autumnalis*) CAA80660; chinook salmon (*Oncorhynchus tshawytscha*) AAB28216; chum salmon (*Oncorhynchus keta*) CAA45407; Atlantic salmon (*Salmo salar*) CAA59258; rainbow trout (*Oncorhynchus mykiss*) AAA49611; stinging catfish (*Heteropneustes fossilis*) AAK53436; channel catfish (*Ictalurus punctatus*) AAF82287; Japanese eel (*Anguilla japonica*) AAO17792; European eel (*Anguilla anguilla*) CAA48902.

2.2. RT-PCR analyses: sex-specific tissue expression

Tissues were collected from adult yellow perch kept in (IACUC #00251L2001) approved aquaculture facilities at the University of Kentucky, Lexington, KY, USA. Perch were kept in a flow through tank (∼1 L/min) chilled to ∼17 °C on a 14:10 light:dark cycle with constant aeration. Fish were kept at a density of <1 fish/3.8 L and fed to satiation every other day with Aquamax Grower 400 (PMI Nutrition International, Inc., Brentwood, MO) for a period of one year before sampling. Three male and three female yellow perch were sampled in spring (March) and euthanized with MS 222 before tissues were harvested. Brain, pituitary, gill, heart, liver, stomach, spleen, kidney, skeletal muscle and gonad tissues were collected along with post-vitellogenic oocyte tissue. Tissues were collected and flash frozen at −80 °C until analysis. Total RNA was extracted as previously described, quantified by UV spectrophotometry and RNA quality was assessed by agarose gel electrophoresis and ethidium bromide staining. Transcript sizes of pituitary

mRNAs were also verified with northern blotting using the DIG Northern Starter Kit™, according to the manufacturer's protocol (Roche Applied Sciences, Indianapolis, IN, USA). Equal amounts of RNA from each individual were pooled by sex-specific tissue. Total RNA was treated with amplification grade DNase, quantified and up to 750 ng was reverse transcribed to cDNA and quantified. For PCR amplification, 900 ng of cDNA template was used, in conjunction with the primers listed in Table 2 and the amplification protocol utilized the same touch-down PCR as described for cloning of the full-length cDNAs. A no-template reaction control (not shown) was also performed with each PCR and the position of the primers in the UTRs minimizes the potential for amplification of genomic fragments. Following PCR amplification, products were electrophoresed in 1% agarose gels and visualized using ethidium bromide staining. cDNA template quality, and quantity (equal loading of material), was verified by analyzing for β-actin mRNA levels using real-time quantitative PCR (qPCR) (data not shown).
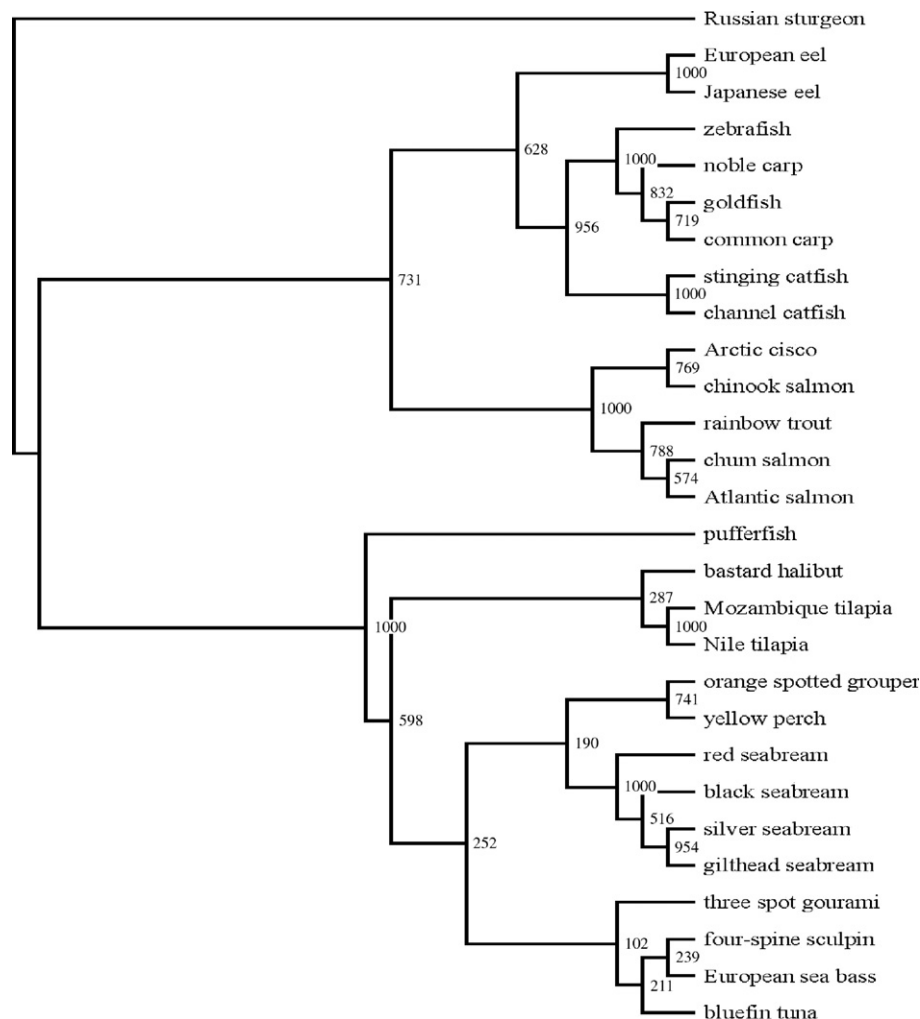
Fig. 3. Rooted phylogenetic tree of mature teleost PRL amino acid sequences. The tree was produced using Clustal X (1.81) alignment and TreeView (Win 32) v. 1.6.6. The tree was rooted with Russian sturgeon (*Acipenser gueldenstaedtii*) PRL (Accession AAB28396) as an outgroup representing the closest available species not in the group Teleostei. See Fig. 2 for EMBI/GenBank Accession numbers for the other sequences used. Numbers at the nodes represent bootstrap values for 1000 trials.

3. Results

3.1. Prolactin

From the sequences generated, yellow perch PRL cDNA (Accession AY332491) was found to consist of 2306 bp containing an open reading frame of 633 bp, a 62 bp 5′-UTR and a 1608 bp 3′-UTR (Fig. 1). The consensus AATAAA polyadenylation signal was located 23 bases upstream of the poly (A) tail. The open reading frame encoded a protein of 211 amino acids, which included a putative signal peptide of 24 amino acids and a mature protein of 187 amino acids. The isoelectric point and molecular mass of the mature PRL peptide, as calculated by Vector NTI BioPlot, are 6.31 and 20,640.56 Da, respectively. Five cysteine residues were present, with only four in the mature peptide and one in the signal peptide. Northern blots verified a single transcript of approximately 2.3 kb in size from total pituitary RNA (data not shown).
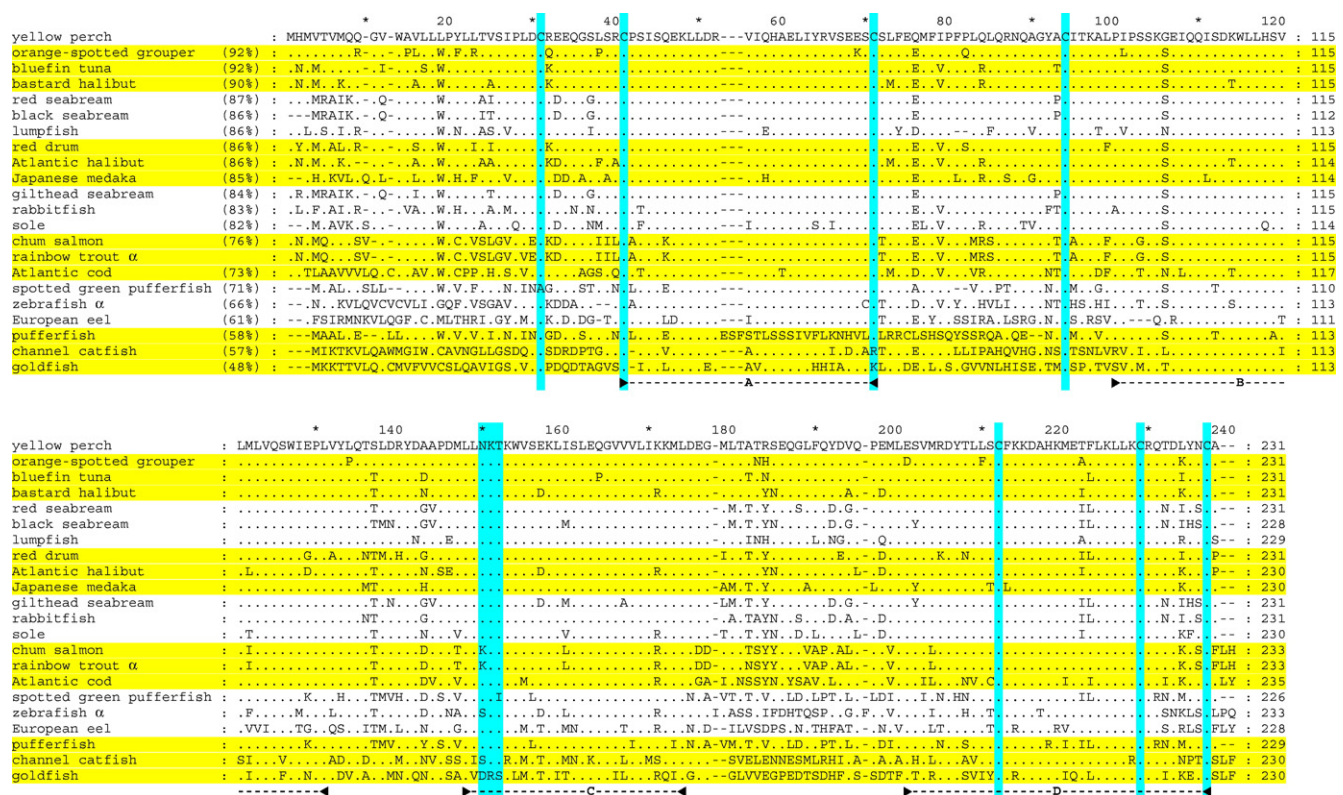
Comparison of the deduced amino acid sequence of the yellow perch PRL with other known teleost PRLs is shown in Fig. 2. After each sequence name, the percent homology (%) with yellow perch PRL, as determined by a BLASTP v. 2.2.14 search (Altschul et al., 1997), is listed. Yellow perch PRL was also determined to have the following homologies with other PRLs: 40% to lungfish; 38% to bullfrog, streamside salamander, African clawed frog and green sea turtle; 37% to horse and rabbit; 36% to human, common quail and chicken; 35% to crocodile, cat, American alligator, mouse and bovine; and 34% to dog and pig. The four cysteine residues in the mature peptide of the yellow perch PRL were conserved throughout the teleost lineage and

have been shown to be involved in the formation of disulfide bonds. Yellow perch was unique in comparison to all other teleosts examined as it was missing an amino acid at position 66 in the comparison sequence (Fig. 2) which corresponds to position 63 in the yellow perch sequence (Fig. 1).

A phylogenetic tree of mature PRL proteins in teleosts (Fig. 3), with sturgeon as an outgroup, was constructed from alignment results. The tree indicates an early divergence of Neoteleostei. The tree also indicates that Salmoniformes (salmon and trout) share a common ancestor with Anguilliformes (eels), Siluriformes (catfish) and Cypriniformes (goldfish, etc.), but they split into their own clade prior to divergence of those other groups. However, there appears to be low branch support for grouping chum salmon and Atlantic salmon as sister groups. The low branch support (bootstrap values) likely reflects the fact that proteins with highly similar amino acid sequences are likely to be grouped in any order (i.e., low bootstrap value) reflecting limitations in assigning implied relationships. Consequently, the PRL amino acid sequences of chum salmon and Atlantic salmon are identical and therefore paired as sister groups in the phylogenetic tree. The branching of rainbow trout as a separate group from the Atlantic salmon and chum salmon can also be attributed to a high sequence homology between these two species, with the exception of a single base substitution of Ile_{209} (I) for Lys (K) (Fig. 2). The PRL amino acid sequence for the Arctic cisco is nearly identical to that of the salmonid sequences with the exception of a single amino acid substitution Val_{148} for Met (present in the cyprinids) which is also present in the chinook salmon (Fig. 2). Given the high number of amino acid sequence substitutions in the

Fig. 4. Nucleotide and deduced amino acid sequences of yellow perch SL cDNA. The nucleotides (lower row) are numbered on the left and the amino acids (upper row) are numbered on the right. The signal peptide is underlined and the seven cysteine residues in the mature protein are shaded. The putative polyadenylation signal is in bold and double underlined and a putative glycosylation site is in bold starting at nucleotide 460. Yellow perch SL cDNA sequence is available from the EMBL/GenBank database with accession AY332490.

Chinook salmon (compared with the other salmonids) and a similar substitution in the Arctic cisco PRL sequence, it is not surprising that these two species are paired as sister groups (Figs. 2 and 3). Within Neoteleostei, the order Tetraodontiformes (pufferfish) splits first, then the families Paralichthyidae (bastard halibut) and Cichlidae (tilapia) are grouped in the next split, despite the former being in the order Pleuronectiformes and the latter being in Perciformes. The families Osphronemidae (three spot gourami), Scombridae (bluefin tuna), Moronidae (European sea bass) and Cottidae (four spine sculpin) split as a group, but subdivide in the above order. While all of those families are in the order Perciformes, with the exception of Cottidae which is Scorpaeniformes, there is low branch support (low bootstrap values) for these groupings and this likely reflects high similarities in PRL amino acid sequences or a lack of additional PRL sequences for fishes within these families. Next Serranidae (orange spotted grouper) and Percidae (yellow perch) split before the emergence of Sparidae (seabreams), but the bootstrap value for this node/split is low, despite higher values for the specific groupings. Again, the low support for this split is likely due to sequence similarities among these groups and to the lack of additional sequence information for other closely related families.

3.2. Somatolactin

Yellow perch SL cDNA (Accession AY332490) was found to consist of 1589 bp containing an open reading frame of 693 bp, a 27 bp 5′-UTR and an 866 bp 3′-UTR (Fig. 4). The consensus AATAAA polyadenylation signal was located 15 bases upstream of the poly (A) tail. The open reading frame encoded a protein of 231 amino acids, which included a putative signal peptide of 24 amino acids and a mature protein of 207 amino acids. The isoelectric point and molecular mass of the mature SL peptide as calculated by Vector NTI BioPlot, are 5.49 and 23,896.39 Da, respectively. Seven cysteine residues were present in the mature peptide and a potential N-glycosylation site was located (Asn_{145}-Lys-Thr_{147}). Northern blots verified a single transcript of approximately 1.6 kb in size from total pituitary RNA (data not shown).

Fig. 5. Alignment of the deduced yellow perch pre-pro-SL amino acid sequence with other teleost pre-pro-SLs. Conserved amino acid residues are indicated with a (.) and inserted gaps are indicated with a (–). The seven mature peptide cysteines and the potential glycosylation site are shaded. Four conserved domains (A_{SL}, B_{SL}, C_{SL}, D_{SL}) are indicated at the bottom of the alignment in bold. The percent listed (%) after the name for each sequence represents the homology with the yellow perch SL sequence as determined by a BLASTP v. 2.2.14 search (Altschul et al., 1997). Sequences were downloaded from the EMBL/GenBank database with the following accession numbers: orange-spotted grouper (*Epinephelus coioides*) AAN18040; lumpfish (*Cyclopterus lumpus*) AAC38004; bluefin tuna (*Thunnus thynnus*) BAE45637; red drum (*Sciaenops ocellatus*) AAD17534; rabbitfish (*Siganus guttatus*) BAA83467; red seabream (*Pagrus major*) BAE43855; black seabream (*Acanthopagrus schlegelii*) AAU43768; gilthead seabream (*Sparus aurata*) CAA72031; sole (*Solea senegalensis*) AAA61873; Atlantic halibut (*Hippoglossus hippoglossus*) AAC38003; bastard halibut (*Paralichthys olivaceus*) AAA49444; Japanese medaka (*Oryzias latipes*) AAT58046; pufferfish (*Tetraodon miurus*) AAF64522; spotted green puffer (*Tetraodon nigroviridis*) AAR25695; Atlantic cod (*Gadus morhua*) BAA01486; chum salmon (*Oncorhynchus keta*) BAA01485; rainbow trout (*Oncorhynchus mykiss*) (Yang et al. 1997); European eel (*Anguilla anguilla*) AAB53035; goldfish (*Carassius auratus*) AAC60098; zebrafish α (*Danio rerio*) AAR25212; channel catfish (*Ictalurus punctatus*) AAF78945.

Comparison of the deduced amino acid sequence of the yellow perch SL with other known teleost SLs is shown in Fig. 5. The conserved amino acid residues of fish SLs cluster in four domains designated A, B, C and D (Company et al., 2000). After each sequence name, the percent homology (%) with yellow perch SL, as determined by a BLASTP v. 2.2.14 search (Altschul et al., 1997), is listed. Yellow perch SL was also determined to have the following homologies with other SLs: 66% white sturgeon; 62% marbled lungfish; and 48% zebrafish β. Of the seven cysteine residues in the mature peptide of the yellow perch SL, five were conserved throughout the teleost lineage and two were conserved in all but three fish (spotted green pufferfish, goldfish and channel catfish). And the potential N-glycosylation site (Asn-Lys-Thr) at alignment positions 150–152 exists in all species except spotted green pufferfish, chum salmon, rainbow trout, goldfish, zebrafish α and channel catfish.

A phylogenetic tree of mature SL proteins in teleosts (Fig. 6), with sturgeon as an outgroup, was constructed from alignment results. The tree indicates an early divergence of the orders

Anguilliformes (eel), Siluriformes (catfish) and Cypriniformes (goldfish etc.). The order Salmoniformes (salmon and trout) splits before the emergence of Neoteleostei. Gadiformes (cod) is the first order within Neoteleostei to emerge followed by Tetraodontiformes (pufferfish) and Pleuronectiformes (sole and halibut). Most of the rest of the fish are in the order Perciformes with the exception of Japanese medaka (Adrianichthyidae) in Beloniformes and lumpfish (Cyclopteridae) in Scorpaeniformes. These two species, along with the families Percidae (yellow perch) and Serranidae (orange-spotted grouper), both of which are in Perciformes, emerge from the remainder of the Perciformes. Next Scombridae (bluefin tuna) and Sciaenidae (red drum) split before Siganidae (rabbitfish) emerges from Sparidae (seabreams). Interestingly, zebrafish SLβ and rainbow trout SLP (somatolactin-like protein) are grouped with European eel, channel catfish and goldfish. It is important to note that the bootstrap values show low support for the branches where Salmoniformes and Neoteleostei diverge. While there is greater support for the maximum likelihood method which
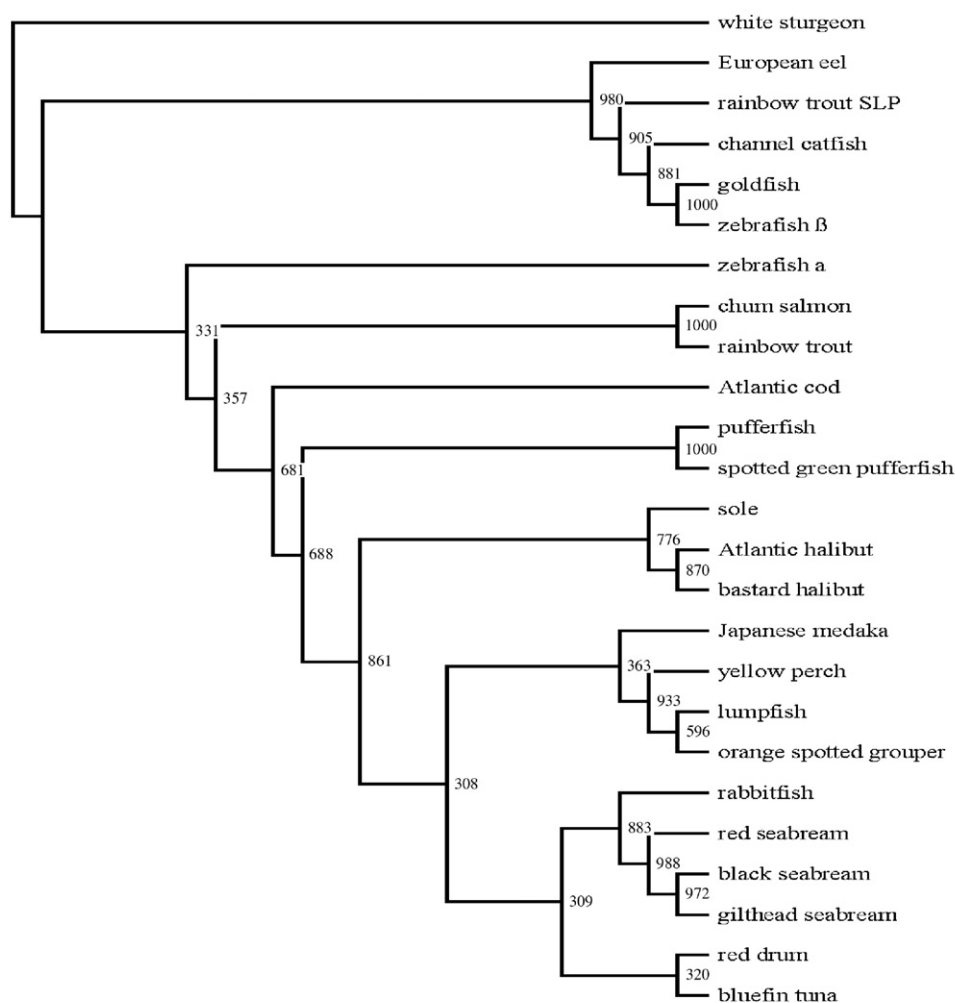
Fig. 6. Rooted phylogenetic tree of mature teleost SL amino acid sequences. The tree was produced using Clustal X (1.81) alignment and TreeView (Win 32) v. 1.6.6. The tree was rooted with white sturgeon (*Acipenser transmontanus*) SL (Accession BAA32608) as an outgroup representing the closest available species not in the group Teleostei. See Fig. 5 for EMBI/GenBank accession numbers except: zebrafish β (*Danio rerio*) NP_001032763; rainbow trout SLP (*Oncorhynchus mykiss*) (Yang and Chen, 2003). Numbers at the nodes represent bootstrap values for 1000 trials.

groups families into sister groups, the low bootstrap values for the early split of Salmoniformes and Cypriniformes, from Neoteleostei, may be due to the occurrence of varying forms of SL (i.e., zebrafish β and α) as well as to having fewer known SL sequences from other teleost families.

3.3. Insulin-like Growth Factor-1

Yellow perch IGF-I cDNA (Accession AY332492) was found to consist of 814 bp containing an open reading frame of 558 bp, a 147 bp 5′-UTR and a 106 bp 3′-UTR (Fig. 7). The open reading frame encoded a protein of 186 amino acids, which included a putative signal peptide of 44 amino acids, an E domain of 74 amino acids and a mature protein of 68 amino acids, consisting of a B domain (29 aa), C domain (10 aa), A domain (21 aa) and a D domain (8 aa). The isoelectric point and molecular mass of the mature IGF-I peptide, as calculated by Vector NTI BioPlot, are 8.31 and 7642.13 Da, respectively. Six conserved cysteine residues, six conserved residues for binding receptors and seven conserved residues for binding IGFBPs were found in the A and B domains of the mature peptide.

Comparison of the deduced amino acid sequence of the yellow perch preIGF-I with other known teleost preIGF-Is is shown in Fig. 8. After each sequence name, the percent homology (%) with yellow perch preIGF-I, as determined by a BLASTP v. 2.2.14 search (Altschul et al., 1997), is listed. Yellow perch preIGF-I was also determined to have the following homologies with other IGF-Is: 60% starlet; 58%

African clawed frog, turkey and chicken; 56% pig, dog, horse, rabbit, bovine and sheep; 55% humans. Of the six cysteine residues in the mature peptide of the yellow perch IGF-I, all were conserved throughout the teleost lineage. Of the six potential receptor binding sites, only the residue at position 124 of the consensus showed any differences and only the orange-spotted grouper had an asparagine instead of a tyrosine. However, all seven of the potential binding protein binding sites were conserved in all known teleosts.

A phylogenetic tree of mature IGF-I proteins (BCAD domains) in teleosts and sterlet (*Acipenser ruthenus*) (Fig. 9) was constructed from alignment results. The tree splits tilapia into its own clade before the divergence of Neoteleostei from more primitive families within Euteleostei (i.e., Cypriniformes, Salmoniformes and Siluriformes). The emergence of tilapia prior to the divergence of Neoteleostei likely reflects a number of unique amino acid substitutions in conserved regions (B-domain: Asn_{90} for Ser; C-domain: Asn_{99} for Ser; D-domain: Ala_{128} for Val, and Ala_{133} and Ala_{134} for Ile and Ser, respectively, which do not occur in other Neoteleosts) that are similar to substitutions occurring in the salmonids, silurids and cyprinids (Fig. 8). After Neoteleostei emerge from the other Euteleostei, the flathead mullet (Mugilidae) splits before the emergence of other families within the order Perciformes. The tree inserts both sculpins (Scorpaeniformes) and the bastard halibut and turbot (Pleuronectiformes) into Perciformes, although it is important to note that the bootstrap values show low support for the branches where these groups diverge. Within Perciformes, the tree groups the seabreams (Sparidae)
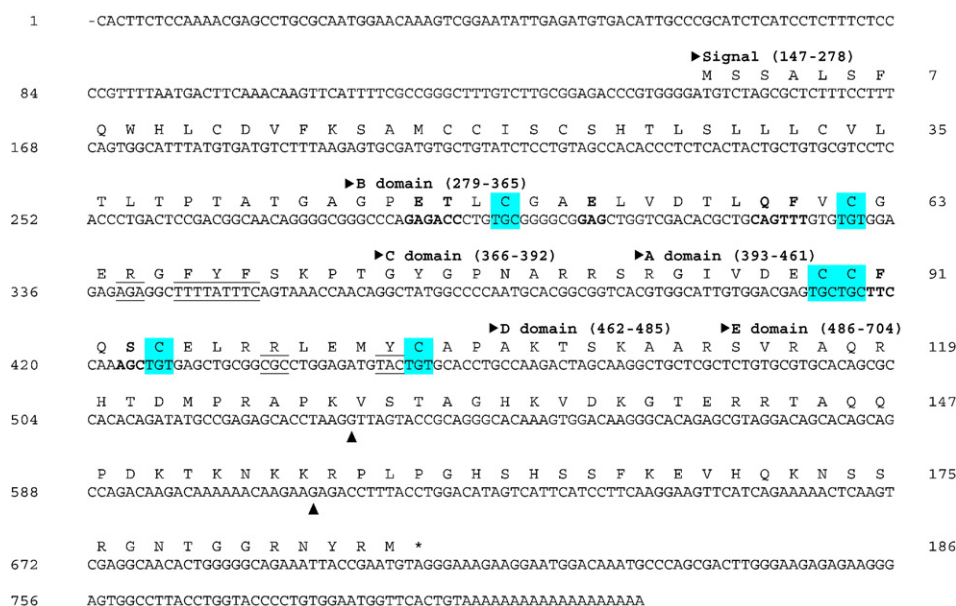

Fig. 7. Nucleotide and deduced amino acid sequences of yellow perch IGF-I cDNA. The nucleotides (lower row) are numbered on the left and the amino acids (upper row) are numbered on the right. The signal peptide and the five structural domains (B, C, A, D and E) are indicated above the amino acids with the respective nucleotide ranges in parentheses. In the B and A domains of the mature peptide the conserved cysteines are shaded, the conserved residues for binding IGF-I receptors are underlined and the conserved residues for binding IGFBP are in bold. The nucleotides between the upward facing bold triangles (▲) represent the region of the E domain missing from the smaller IGF-Ia transcript. The yellow perch IGF-I cDNA sequence is available from the EMBL/GenBank database with accession AY332492.

together and also groups the perch (Percidae) together; however, there is low support for the groupings within Percidae. The low support can be attributed to proteins with highly similar amino acid sequences, which are likely to be grouped in any order (i.e., low bootstrap value), reflecting limitations in assigning implied relationships in the phylogenetic tree anal-

ysis. After the emergence of Neoteleostei, Salmonidae splits and then the channel catfish (Silurifomes) splits from the rest of Teleostei. The fathead minnow (Pimephales) is the first split in the Cyprinidae family and goldfish (*Carassius* spp.) is the second. Instead of grouping the zebrafish of the same genus (*Danio*) as sister groups, the tree grouped the giant danio in with
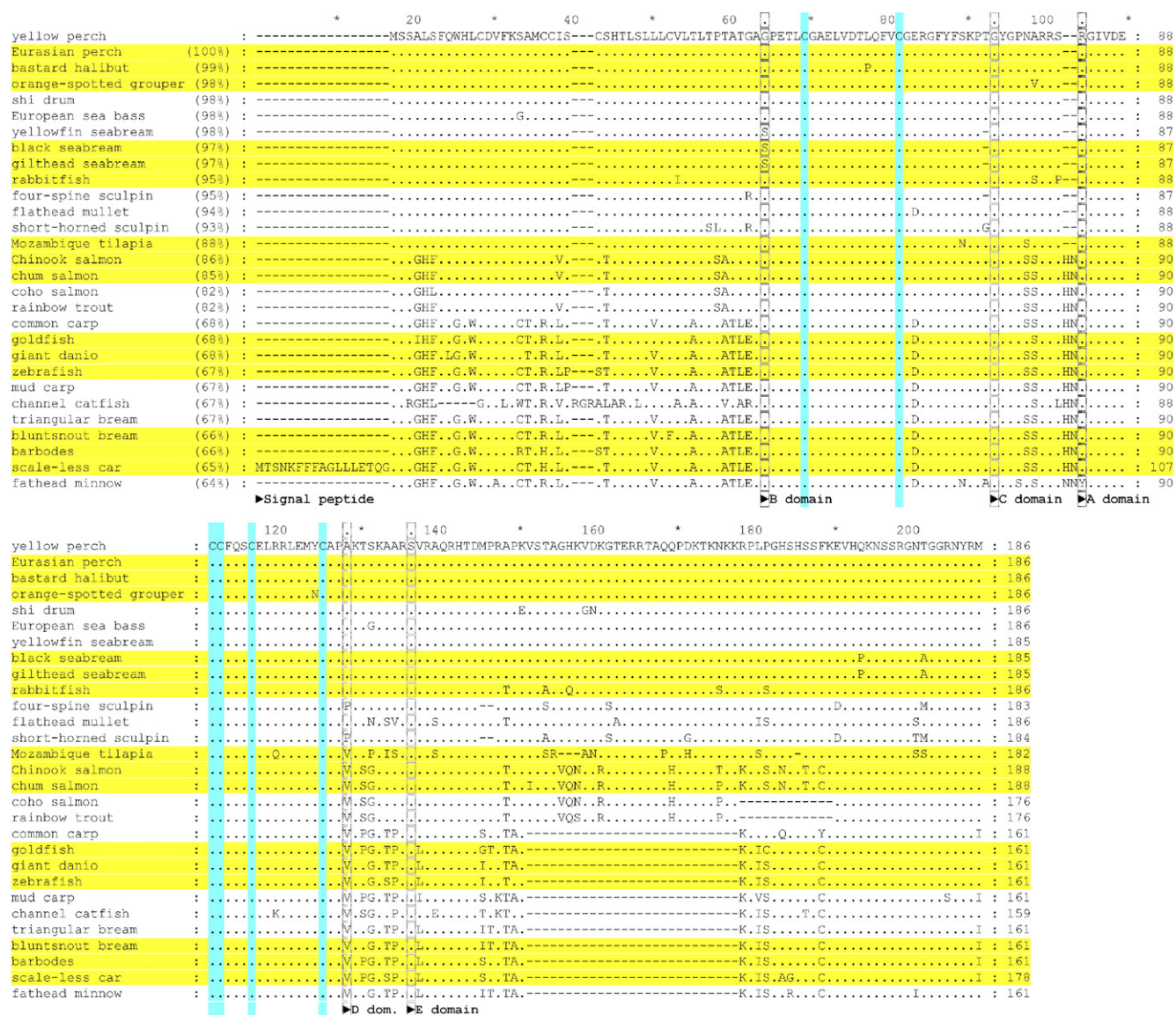
Fig. 8. Alignment of the deduced yellow perch pre-pro-IGF-Ib amino acid sequence with other teleost pre-pro-IGF-Is. Conserved amino acid residues are indicated with a (.), inserted gaps are indicated with a (–) and the six conserved cysteine residues in the mature peptide are shaded. Structural domain start positions (B, C, A, D, E) are identified with □ and at the bottom of the alignment in bold. The percent listed (%) after the name for each sequence represents the homology with the yellow perch IGF-I sequence as determined by a BLASTP v. 2.2.14 search (Altschul et al., 1997). Sequences were downloaded from the EMBL/GenBank database with the following accession numbers: Eurasian perch (*Perca fluviatilis*) CAE52915; bastard halibut (*Paralichthys olivaceus*) CAA09268; shi drum (*Umbrina cirrosa*) AAY21628; black seabream (*Acanthopagrus schlegelii*) AAD01917; gilthead seabream (*Sparus aurata*) AAY46225; yellowfin seabream (*Acanthopagrus latus*) AAT35826; four-spine sculpin (*Cottus kazika*) BAC07249; short-horned sculpin (*Myoxocephalus scorpius*) CAA73162; orange-spotted grouper (*Epinephelus coioides*) AAS01183; rabbitfish (*Siganus guttatus*) AAO47742; European sea bass (*Dicentrarchus labrax*) AAV67967; flathead mullet (*Mugil cephalus*) AAR06903; Mozambique tilapia (*Oreochromis mossambicus*) AAC17494; chinook salmon (*Oncorhynchus tshawytscha*) AAA67263; chum salmon (*Oncorhynchus keta*) AAC18833; rainbow trout (*Oncorhynchus mykiss*) AAA49412; coho salmon (*Oncorhynchus kisutch*) AAA49410; fathead minnow (*Pimephales promelas*) AAT02176; goldfish (*Carassius auratus*) AAC83443; triangular bream (*Megalobrama terminalis*) AAO89239; giant danio (*Danio aequipinnatus*) ABB05519; bluntsnout bream (*Megalobrama amblycephala*) AAK16727; zebrafish (*Danio rerio*) AAI14263; scale-less carp (*Gymnocypris przewalskii*) AAX21106; mud carp (*Cirrhinus molitorella*) AAY21902; common carp (*Cyprinus carpio*) BAA11878; barbodes (*Spinibarbus sinensis*) ABE03747; channel catfish (*Ictalurus punctatus*) AAZ28918.

the scale-less car (*Megalobrama* spp.), for which there is low branch support for this sister grouping. The reason for this is unclear, but a likely explanation may reside in the occurrence of identical amino acid sequences (among the zebrafish, giant danio, triangular bream and blunt-snout bream) in the B, C and A domains, where in the D-domain both the zebrafish and scale-less car share a single amino acid substitution (Thr_{133} to Ser) which is not present in any of the cyprinids examined. To further evaluate this, we performed a similar analysis using the pre-pro-IGF-I proteins (Signal-BCAD-E domains) and differences were noted. More specifically, there were two main branches emerging from the (root) sterlet (*Acipenser ruthenus*) with one branch consisting of Cypriniformes and Siluriformes (Teleostei) and the second consisting of Salmoniformes (Teleostei) and Perciformes (Neoteleostei) (data not shown). Here, the grouping of salmonids with percids is not unexpected as the cyprinids and channel catfish (Siluridae) all possess a significant deletion in the E-domain (amino acid positions 151–

176) of the pre-pro-IGF-I protein, which is not present in the other groups examined (Perciformes and Salmoniformes) (Fig. 8).

3.4. Tissue- and sex-specific expression

The primers in Table 2 were used to generate full length coding region PCR products whose identities were confirmed by DNA sequencing. The GH product was 678 bp in length and corresponded with the published GH EMBL/GenBank sequence (Accession AY007303) (Roberts et al., 2004) while the PRL and SL products were 694 and 767 bp, respectively. The IGF-I primers generated two PCR products that were confirmed by DNA sequencing. The larger product (termed IGF-Ib) (Jentoft et al., 2004) was 620 bp in length and corresponded to the sequence presented in Fig. 7, while the smaller product (termed IGF-Ia) was 539 bp in length and was missing an 81 nucleotide region from the E domain (nucleotides 531–611 in
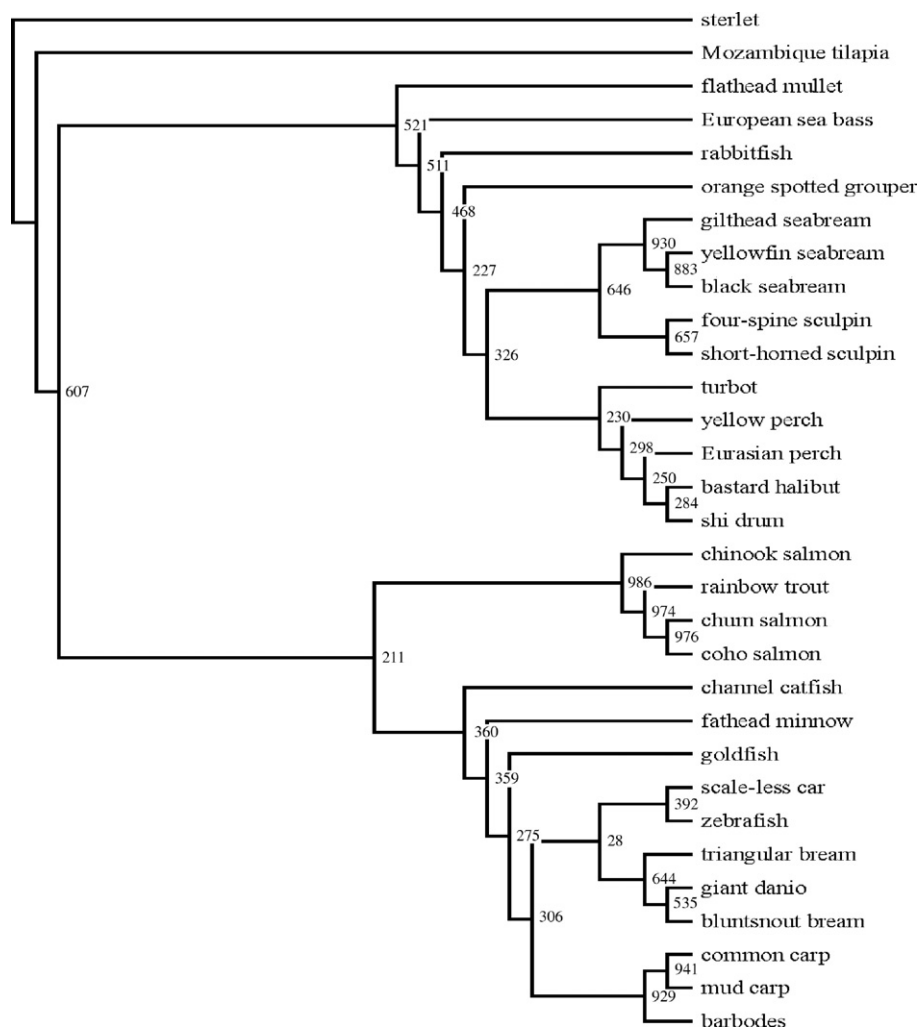
Fig. 9. Rooted phylogenetic tree of mature teleost IGF-I amino acid sequences. The tree was produced using Clustal X (1.81) alignment and TreeView (Win 32) v. 1.6.6. The tree was rooted with sterlet (*Acipenser ruthenus*) IGF-I (Accession ABC54785) as an outgroup representing the closest available species not in the group Teleostei. See Fig. 8 for EMBI/GenBank accession numbers except: turbot (*Scophthalmus maximus*) (Duval et al., 2002). Numbers at the nodes represent bootstrap values for 1000 trials.
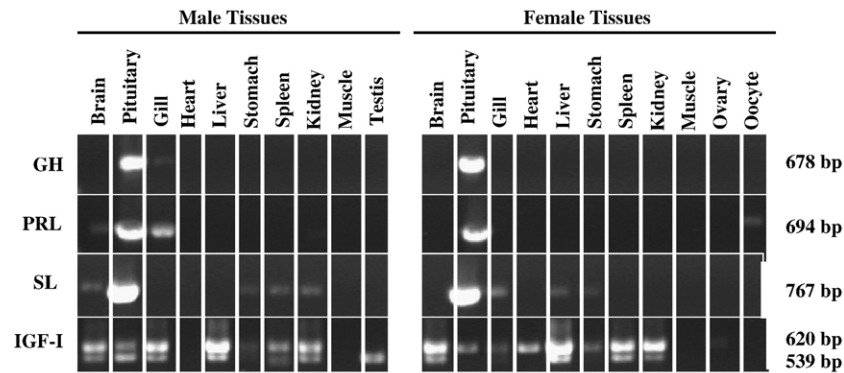
Fig. 10. Sex- and tissue-specific expression of GH, PRL, SL and IGF-I in male and female yellow perch. For PCR primers used see Table 2 and bp sizes of products are shown to the right of the figure. Template used was 900 ng of cDNA generated from the pooled mRNA of three adult yellow perch for each sex-specific tissue. PCR products were then run on a 1% agarose gel and visualized using ethidium bromide staining. cDNA template quality was verified by analyzing for β-actin mRNA levels using real-time quantitative PCR (qPCR) (data not shown). Identity of the products was confirmed via DNA sequencing of the PCR products from several different runs.

Fig. 7). These primers were then used to determine sex- and tissue-specific expression (Fig. 10).

GH, PRL, and SL all showed the highest level of expression in pituitary tissue for both sexes. Male gill tissue had low levels of GH expression, but otherwise there was no extrapituitary expression of GH detected. Male gill tissue showed substantial PRL expression and male brain tissue showed low PRL expression. No female tissues, besides pituitary, showed any PRL expression, however post-vitellogenic oocyte tissue did show PRL expression, presumably from maternally derived transcripts. Male brain, stomach, spleen and kidney tissues showed expression of SL, while female gill, liver and stomach tissues showed SL expression. While the two IGF products showed tissue and sex-specific differences in expression, both sexes showed the highest expression of IGF-Ib in liver tissue. Male brain, pituitary, gill, liver, spleen and kidney tissues showed moderate expression of both IGF-I products. Male stomach tissue showed low levels of expression of both IGF-Is and male testis tissue only showed the IGF-Ia product. Female brain, liver, spleen and kidney tissues also showed moderate expression of both IGF-I products. However, female pituitary, gill, heart and stomach tissues showed moderate expression of only the IGF-Ib product and female ovary tissue showed low expression of only the IGF-Ib product.

4. Discussion

In this study, the full length cDNA sequences for yellow perch PRL, SL and IGF-I have been characterized, phylogenetic analyses conducted and presented on the deduced amino acid sequences and sex- and tissue-specific gene expression patterns determined. PRL cDNA of the yellow perch was cloned by directional RACE procedures and was found to encode a protein of 211 amino acids. Based on the sequence homology analysis, the mature PRL protein contains 187 amino acids, which is similar to the size of most teleost PRLs. This mature PRL, however, is one amino acid residue smaller than the six closest neoteleost species as it lacks an amino acid at position 66 of the alignment (Fig. 2) which, in 23 of the 26 known teleost species,

is a methionine. The significance of this absent amino acid residue remains unknown, as protein activity and function cannot be determined until the natural peptide is isolated and characterized. This minor difference in sequence is unlikely to produce dramatic effects in function as the mature peptide maintains the four conserved cysteines that are responsible for the two disulfide bonds that result in protein folding (Freeman et al., 2000). Amino acid alignment and phylogenetic analysis indicate that yellow perch PRL shares the highest degree of homology with other members of the order Perciformes, such as the orange-spotted grouper and the four seabream species (silver, gilthead, black and red).

Using RT-PCR a single PRL transcript was detected and repeated sequencing efforts produced a single nucleotide sequence. These results suggest that only one form of PRL is present in the pituitary of yellow perch as is the case with most teleosts. The most notable exceptions are the Nile tilapia (O. niloticus) (Rentier-Delrue et al., 1989) and Mozambique tilapia (O. mossambicus) (Specker et al., 1985) which have two distinct forms of PRL. The first is $tPRL_{188}$ which is very similar to other teleost PRLs ($\sim 70\%$) and the second is $tPRL_{177}$ which is 11 amino acids shorter and has much lower homology to other teleost PRLs ($\sim 55\%$) (Yamaguchi et al., 1988). Both are functional prolactins with different activities, at least in regards to osmoregulation (Sakamoto et al., 1997; Auperin et al., 1994). There was no N-glycosylation site found in the yellow perch PRL, whereas one N-glycosylation site was reported in the seabream PRL, resulting in a higher molecular weight compared to other teleost PRLs (Santos et al., 1999).

The full length SL cDNA of the yellow perch was cloned by directional RACE procedures and encodes a protein of 231 amino acids. Based on the sequence homology analysis, the mature SL protein contains 207 amino acids, which is similar to the size of most teleost SLs. The mature yellow perch SL contains seven cysteines, six of which are required for the three intra-peptide disulfide bonds (Rand-Weaver et al., 1991). The cysteines at consensus positions 31 and 41 form the first bond and the last four cysteines are responsible for the other two bonds. Only the spotted green pufferfish lacks one of these important structural cysteines by having an alanine (Ala) at consensus position 31 and is one of

the six species (listed in Fig. 5) that does not contain the conserved *N*-glycosylation site at consensus positions 150–152. Some species such as the rainbow trout (Yang et al., 1997) may only have a non-glycosylated form of SL, however both glycosylated and non-glycosylated forms of SL were secreted from *in vitro* cultures of pituitaries from gilthead seabream and sole (Pendon et al., 1998).

The low homology of the published goldfish SL sequence (Cheng et al., 1997) to yellow perch SL (48%) and most other teleost SLs, could perhaps indicate the existence of a second form of SL (β) in this species, as was recently discovered in zebrafish (Zhu et al., 2004) and is reportedly being investigated in common carp (Company et al., 2000). Interestingly, rainbow trout SLP (somatolactin-like protein) (Yang and Chen, 2003) grouped with zebrafish β, goldfish and channel catfish SL instead of with rainbow trout and chum salmon SL (Fig. 6). Apparently neither zebrafish SL sequence (α or β) contains a potential *N*-glycosylation site (Zhu et al., 2004), but rainbow trout somatolactin-like protein (SLP), and not rainbow trout SL, does contain the conserved *N*-glycosylation site which is present in almost all teleosts (Fig. 5). The presence of SL paralogues (α and β) in some fish species raises some interesting questions on evolution in the Actinopterygii (ray-finned fish). While zbSLβ, rtSLP, goldfish SL and channel catfish SL seem to be orthologs of each other and paralogues of the SLα (Zhu et al., 2004) (Fig. 6), it remains unknown if more species have a second SL. With the SLβ form showing up in two separate families of fish (i.e. Cyprinidae and Salmonidae), it might be expected that more fish species will have a second SL. The phylogenetic trees of PRL (Fig. 3) and IGF-I (Fig. 9) both indicate an early split of Neoteleostei from the other teleosts, while the SL phylogenetic tree (Fig. 6) shows the more ancestral species "peeling off" before the emergence of Neoteleostei. There are very few known SL sequences for non-Neoteleostean fishes and more data may reveal a phylogenetic pattern for SL similar to PRL and IGF-I as well as more species with a SLβ gene.

The full length IGF-I cDNA of the yellow perch was cloned by directional RACE procedures and encodes a protein of 186 amino acids. Based on the sequence homology analysis, the mature IGF-I protein contains 68 amino acids, which is similar in size to most fish IGF-Is. The families Cyprinidae, Ictaluridae and Salmonidae possess histidine (His) and asparagine (Asn) residues at consensus positions 103 and 104, respectively (Fig. 8), while all Neoteleostei lack these residues. The mature yellow perch IGF-I has six conserved cysteine residues with two in the B domain and four in the A domain. These cysteines are conserved in the vertebrate genome (Duval et al., 2002) and are involved in generating the tertiary structure of the mature IGF-I protein. In the mature yellow perch IGF-I peptide the putative disulfide bonds would be: Cys_6-Cys_{46}; Cys_{18}-Cys_{59}; and Cys_{45}-Cys_{50} (Narhi et al., 1993). The greatest conservation in amino acid residues occurs in the B and A domains (86–97%), while considerably less conservation is observed in the C and D domains. The importance of the high sequence identity in the A and B domains lies not only with the maintenance of tertiary structure by cysteine disulfide bonds but also in the binding of IGF-I with its receptor and IGF-binding proteins (IGFBPs) (Fig. 8). Studies done in mammalian systems

show residues involved with binding of mature IGF-I with its receptor include Arg_{21} and the Phe_{23}-Tyr_{24}-Phe_{25} motif in the B domain and Arg_{54} and Tyr_{58} in the A domain (Bayne et al., 1990), while residues involved in IGF-I binding with IGFBP include Glu_3, Thr_4, Glu_9, Gln_{15} and Phe_{16} in the B domain and Phe_{47} and Ser_{49} in the A domain (Clemmons et al., 1992). Conserved residues in the C and D domains have also been reported to be important in hIGF1R binding, such as C domain Tyr_{31} and Arg_{36}-Arg_{37} residues and D domain Lys_{63} and Lys_{66} residues (Bayne et al., 1990).

In yellow perch, sex- and tissue-specific expression, as determined by RT-PCR, reveals that the pituitary gland tissue is the primary expression site for GH, PRL and SL (Fig. 10). Male gill tissue showed low levels of GH and moderate levels of PRL expression which is consistent with their roles in osmoregulation (Sakamoto and McCormick, 2006). However female gill tissue did not show GH or PRL expression, but instead showed moderate levels of SL expression. While other studies have found SL expression in gill tissue (Yang et al., 1997), a functional role for gill SL and the significance of a sex-specific expression pattern of SL in yellow perch gill tissue remains unclear. In coho salmon, male and female gill tissues showed moderately high levels of SLR expression (equivalent with pituitary tissue) which raises the possibility that gill SL expression could have an autocrine function separate from SL's pituitary endocrine function (Fukada et al., 2005). Another example of a sexually dimorphic tissue expression pattern in yellow perch is the PRL and SL expression in brain tissue. Male brain tissue shows low expression levels of both PRL and SL while female brain tissue does not show any expression. SL also exhibited low expression levels in male stomach, spleen and kidney tissues and female liver and stomach tissues. In rainbow trout, GH, PRL and SL were all detected in mature oocytes presumably as maternal messages (Yang et al., 1999). IGF-I has similarly been detected in mature oocytes of Mozambique tilapia (Kajimura et al., 2004) but only PRL, and not GH, SL or IGF-I, was expressed in yellow perch post-vitellogenic oocyte tissue. These oocytes were collected in March and are post-vitellogenic, but characterized as being in pre-final maturation as described in Dabrowski et al. (2002) and Ciereszko et al. (1997). The PRL messages detected are presumably maternal in nature, suggesting that PRL may be involved in oocyte maturation in yellow perch.

IGF-I shows a very global expression pattern in both male and female yellow perch and tissue expression analysis reveals two IGF-I bands, corresponding to 620 bp (IGF-Ib) and 539 bp (IGF-Ia). These results indicate the presence of two separate yellow perch IGF-I mRNA transcripts which correspond directly with Eurasian perch IGF-Ib and IGF-Ia (Jentoft et al., 2004). The two transcripts of IGF-I in Eurasian perch are generated by alternative splicing of the pre-mRNA and Eurasian perch IGF-Ib has 100% homology with yellow perch IGF-Ib (Fig. 8). Both yellow and Eurasian perch IGF-Ia cDNAs are missing 81 nucleotides from the E domain which correspond to 27 amino acids starting with Val_{129} and ending with Lys_{155} (Fig. 7). The Eurasian perch IGF-Ia and IGF-Ib cDNAs also correspond to the two IGF-I transcripts identified in Japanese flounder (Tanaka et al., 1998) and barramundi (Ståhlbom et al., 1999). The E domain of IGF-I is

highly variable and four different transcripts have been found in several salmonids which are designated IGF-I Ea-1, Ea-2, Ea-3, and Ea-4 (Duguay et al., 1994). The four transcripts are derived from two separate duplicated IGF-I genes (Kavsan et al., 1994) with two alternatively spliced transcripts from each duplicated gene (Wallis and Devlin, 1993). Yellow perch IGF-Ib shows high homology to coho salmon Ea-4 (Fig. 8) and Eurasian perch IGF-Ia and the second yellow perch transcript (IGF-Ia) correspond with salmon Ea-2 (Duguay et al., 1994). This would indicate the two IGF transcripts in yellow and Eurasian perch are alternatively spliced transcripts from a single gene and not derived from two duplicate genes. Interestingly, in salmon liver, the Ea-2 transcript was not detected and only Ea-1 and Ea-3, not Ea-4, responded to GH treatment.

There was a clear sex-specific expression pattern with IGF-I in yellow perch non-hepatic tissues. All male yellow perch tissues that expressed IGF-I expressed both transcripts, with the exception of testis tissue, but female yellow perch tissues in many cases expressed only IGF-Ib. However, yellow perch testis tissue only expressed the IGF-Ia transcript and yellow perch ovary tissue had very low levels of expression of only IGF-Ib (Fig. 10). Several studies have shown that the E-peptides of pro-IGF-I posses novel biological activities, such as controlling malignant properties of cancer cells *in vitro* (Chen et al., 2002), stimulation of mitogenic activity in several cell lines (Tian et al., 1999) and regulation of cell growth and differentiation (Kuo and Chen, 2002). Consequentially, the difference in sex-specific tissue expression of the two IGF-I transcripts may suggest that the E peptides in this species also possess biological activities perhaps even related to sexual function.

In conclusion, this study provides the full length nucleotide sequences for yellow perch PRL, SL and IGF-I cDNAs and shows that there is a high degree of similarity with other fish species at the amino acid level. Yellow perch GH, PRL and SL all showed only one transcript and significantly higher levels were observed in the pituitary as compared with other tissues. IGF-I, however, showed two transcripts (designated IGF-Ib and IGF-Ia) which showed distinct sex-specific tissue expression differences. These results indicate new avenues for research related to extra-pituitary expression of these genes and the sex-specific tissue expression of IGF-I transcripts. The production and publication of these yellow perch sequences provides molecular tools to investigate the endocrine basis for the sexual size dimorphism (growth) in yellow perch as well as other physiological sectors in this teleost species.

Acknowledgements

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